IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

APPLICANT(S)	:	Page W. Caufield *et al*	ART UNIT	: 1645
SERIAL NO.	:	10/672,784	EXAMINER	: Hines, Jana A.
FILED	:	September 26, 2003		
FOR	:	ENHANCED PRODUCTION OF STREPTOCOCCUS MUTANS I AND III		

Certificate of Mailing Under 37 CFR 1.8

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Loretta Kavanagh (Name of Depositor)

Loretta Kavanagh 2/27/2007 (Signature and Date)

DECLARATION UNDER 37 C.F.R. 1.132

COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, VA 22313-1450

Dear Sir:

I, Zenggo He, Ph.D. as evidenced by my signature below, declare the following:

1. I received my Ph.D. in Microbiology in 1996 from the China Agricultural University, College of Biological Science, Beijing, China. I received my M.S. in Microbiology in 1991 from China Agricultural University (formerly Beijing Agricultural University), College of Biological Sciences, Beijing, China. I received my B.S. in Microbiology in 1986 from Beijing Agricultural University College of Biological Sciences, Beijing, China.

2. Since October of 2003 I have been working as a Research associate 2-B/H in Food Science and Technology at the Ohio State University in Columbus, Ohio primarily in scaling up

of the production and purification of novel lantibiotic paenibacillin.

3. From January, 2002 until September, 2003 I worked as a Research Scientist in Bacterial Physiology and Fermentation Facility Lab at the New York University in New York City where I was in charge of the fermentation facilities and scaling up of mutacin I and III production.

4. From May, 2000 until December, 2001, I was a Postdoc and Research associate in the Department of Oral Biology at the University of Alabama at Birmingham, Birmingham, Alabama where I worked primarily on a submerged fermentation process for the production of antimicrobial peptide mutacins.

5. From June, 1998 until April, 2000 I was an investigator in the Department of Biotechnology, CINVESTAV, Mexico City, Mexico where I worked primarily in investigating microcycle conidiation in *Paecilomyces fumosoroseus.*

6. From July, 1995 until May, 1998 I was a lecturer and asisstant professor at the China Agricultural University/Henan SanBao (Group) Share Co, Ltd., Beijing, China where I worked primarily in scaling up GA4+7 fermentation sequentially from 30, 300, 3000 liter to a final 30,000 liter industrial scale. Prior to that I held other research positions.

7. I have reviewed the above-noted application serial number 10/672,784 and the claimed subject matter corresponding to the currently pending claims in the application.

8. Attached as Exhibit A to this Declaration is a copy of my curriculum vitae.

9. Previously, four mutacins were found in the laboratory by different *Streptococcus mutans*. Mutacin I, II and III are lantibiotics whereas mutacin IV is not. Mutacin I and III have a stronger antimicrobial potency as compared with mutacin II and V.

10. Techniques for submerged production of mutacin II had been successfully worked out, by using media applying glucose as the carbon source. However, when the same conditions were used for mutacin I and III in submerged culture, no obvious production was observed. Thus before the invention invention, submerged production of mutacin I and III was not possible. The instant invention is directed to methods for successfully producing the same in a liquid culture. One of the key factors that allows producing mutacin I and III is the use of sucrose.

11. It is reported that one of the most crucial conditions for mutacin I and III is cell density. In solid culture, the producer cells form into bio-film based colonies. Thus, mutacin I and III can be produced in any media tested. However, in liquid culture, reaching a biofilm based high cell density is a huge challenge. This is clarified by the unexpected failure to produce mutacin I and III when media having glucose as the carbon source are used.

12. The presently claimed methods overcome this difficulty by using an OPM medium having sucrose as the carbon source. This medium allows exopolysaccharide production and enables the producer cells to aggregate together and form into pellets (*See*, Figure 1, submitted herewith). Thus, the present invention produces biofilm based high cell density (*See*, Figure 2, submitted herewith). As a result, a high yield of both mutacins I and III in submerged culture is attaned. A cell pellet is never observed in a media having glucose as the carbon source (See, Figure 3, submitted herewith).

13. All statements made herein of my own knowledge are true and all statements made on information and belief are believed to be true; and further these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code; and that such willful false statements may jeopardize the validity of the application, or any patent issuing thereon.

Submitted by

_____ Date: _____, 2007

Zenggo He, Ph.D.

CURRICULUM VITAE

ZENGGUO HE, Ph.D.

RESIDENCE:
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EXPERTISES SUMMARY

- Over 10-year research and industrial experience with recognized establishment in microbial product development (both fungal and bacterial), as well as upstream optimization & scale up, down stream separation and purification
- Fresh experience with purification and production of microbial metabolites from small antibiotic to bioactive lantibiotic peptide by microbial fermentation, probiotic bacteria cultivation & anaerobic fermentation
- Expertise with process scale up from bench level to pilot & industrial level
- Familiar with down stream operation techniques like TFF, lyophilization and large scale chromatography for small metabolite and protein purification;
- Solid knowledge with techniques for small secondary metabolite and protein purification and characterization (HPLC, LC, GC, GC-MS, LC-MS, MALDI-MS, MS/MS, NMR)
- Familiar with molecular and genetic tools for strain screening and identification

MAIN SCIENTIFIC ACHIEVEMENTS

2003-2006	Discovered a novel lantibiotic (paenibacillin) which has a broad antimicrobial spectrum and is potent for food and medical application
2000-2003	Established the first set of techniques in the world for the manufacture of lantibiotics mutacin I and III in liquid culture
1993-1998	Developed a novel fermentation product GA4/7, which bridged the gap of Chinese gibberellin industry, by scaling up from bench level to industrial scale
1997-1998	Compiled the first two manufacture standards for industrial gibberellin production in China

ACADEMIC EXPERIENCES

10/2003-present Research associate 2-B/H, Food Science and Technology, the Ohio State University, Columbus, Ohio

RESEARCH ACTIVITIES:

- Scale up of the production and purification of novel lantibiotic paenibacillin
- Fermentation optimization and Scale up of the production of panibacillin
- Genetic analysis of gene cluster involved in paenibacillin biosynthesis
- Study on the biosynthesis of antibiotic polymyxin, its purification and down stream recovery
- Purification of novel panibacillin by FPLC and HPLC
- Characterization of novel antimicrobials by LC-MS, MALDI-MS, MS/MS and NMR
- Study on the probiotic bacterium *Lactobacillus casei* cultivation and fermentation
- Screening for GRAS microbes (e.g., lactic acid bacteria) that have potential for bio-preservation by traditional and molecular methods
- Identification of GRAS microbes by 16S rDNA techniques and biochemical methods
- Heterologous expression of antimicrobial peptide by Lactic Acid Bacteria (LAB)

01/2002- 09/2003 Research scientist, Bacterial Physiology and Fermentation Facility Lab, New York University, New York

RESEARCH ACTIVITIES:

- In charge of the fermentation facilities and scale up of mutacin I and III production
- Production, purification of antimicrobial peptide mutacin by lactic acid bacterium *Streptococcus mutans*
- Computer controlled Fermentation process optimization by AFS-BioCommand Bioprocess Software
- Mutacin antibodies production, ELISA test of mutacin antibodies
- Isolation, purification of exopolysaccharide from the culture of *Streptococcus mutans*
- Study on the killing mechanism of mutacin III (patch-clamp analysis)

05/2000-12/2001 Postdoc and Research associate, Department of Oral Biology, Univ. of Alabama at Birmingham, Birmingham, Alabama

RESEARCH ACTIVITIES:

- Submerged fermentation process for production of antimicrobial peptide mutacins
- Developed methods for down stream recovery of antimicrobial peptide mutacins from fermentation broth
- Optimizing media for mutacins production

- Study on the physiology of mutacins production by *Streptococcus mutans*
- Functional studies of the activity of mutacins by protein engineering techniques

06/1998-04/ 2000 2-A investigator, Department of Biotechnology, CINVESTAV, Mexico City, Mexico

RESEARCH ACTIVITIES:
- Investigating microcycle conidiation in *Paecilomyces fumosoroseus*
- Developed methods for submerged conidiation by *Paecilomyces fumosoroseus*
- Optimizing media for submerged conidiation by *Paecilomyces fumosoroseus*
- Developed methods for conidia production by *Thichoderma harzianum*

07/1995-05/1998 Lecturer/asisstant professor, China Agricultural University/Henan SanBao (Group) Share Co, Ltd., Beijing, China

RESEARCH ACTIVITIES:
- Scaled up GA4+7 fermentation sequentially from 30, 300 3000 liter to a final 30,000 liter industrial scale
- Developed a double solvent system for the down-stream purification of GA4+7
- Study on the regulation of GA4+7 biosynthesis
- Established the reverse-phase HPLC method for the separation and quantitative analysis of gibberellins mixture

09/1992-09/1995 Associate investigator, NECAE, Beijing, China

RESEARCH ACTIVITIES:
- Study on the inhibition of NH4+ on the biosynthesis of gibberellins
- Study on the affect of other secondary metabolites produced by *Fusarium monilifome* Sheld H17 on the biosynthesis of gibberellins
- Study on the regulation of gibberellins biosynthesis

09/1989-08/1992 Assistant investigator NECAE, Beijing, China

RESEARCH ACTIVITIES:
- Screening for high yield gibberellin-producing strain
- Guiding GA3 production, Qi-He and Xin-Du Fermentation Plants

INDUSTRIAL EXPERIENCES

07/1995-05/1998 Project director, Henan SanBao (Group) Share Co, Ltd., Beijing, China

DUTIES AND ROLES:
- Scaled up GA4+7 fermentation sequentially from 300, 3000 liter to a final 30,000 liter industrial scale

- Developed a double solvent system for the down-stream purification of GA4+7
- Study on the regulation of GA4+7 biosynthesis
- Established the reverse-phase HPLC method for the separation and quantitative analysis of gibberellins mixture
- Toxicity test and product stability test

09/1993-09/1995 Scientific mentor, San-Ming Company, Beijing, China

DUTIES AND ROLES:
- Guiding the production of a fungal lipid, γ-linolenic acid
- Guiding the analysis and purification of γ-linolenic acid

09/1989-09/1995 Associate investigator/ engineer, Pilot plant of National Experimental Center for Agromicrobiological Engineering (NECAE), Beijing, China

DUTIES AND ROLES:
- Scaled up GA4+7 fermentation sequentially from flask level to 30 and 100 liter scale
- Analysis of GA fermentation broth by GC-MS

09/1987-08/1988 Technical support engineer in fermentation plants in Sichuan and Shandong provinces, China

DUTIES AND ROLES:
- Guiding GA3 production, Qi-He and Xin-Du Fermentation Plants

TEACHING EXPERIENCES

1992-1998 Lecturer/assistant professor, China Agricultural University, Teaching Industrial Microbiology, General Microbiology and Bacteriology

RESEARCH GRANTS AND ACADEMIC AWARDS

1999 Reward for the Invention of High-tech Product GA4+7 by the Scientific Committee of Henan Province, China

1997 Reward for Important Scientific Achievement, Scientific Committee of China

1995 Reward for Breakthrough of Chinese Gibberellins Industry, Scientific Research Foundation of China

1991 Reward for Outstanding Scientific Research, Ministry of Agriculture of China

PATENTS

- Page Caufield and Zengguo He. Enhanced Production of Streptococcus mutant mutacin I and III production. US Patent 2004146983
- Ahmed Yousef, Zengguo He Chunhua Yan, Liwen Zhang and Duygu Kisla. Antibiotic antimicrobial peptide agents and methods of their use (filed)
- Ahmed Yousef, Zengguo He, Chunhua Yan and Liwen Zhang. ANTIBIOTIC ANTIMICROBIAL AGENTS AND METHODS OF THEIR USE (filed)

BOOK CHAPTERS

He Zengguo Improving apple quality by spray of GA4+7. In: The guidebook of Applicable Techniques for Little to Medium Cities. Chinese Scientific Press, Beijing 1997

JOURNAL ARTICLES

1. **Yan Fanggui, He Zengguo and Li Jilun.** (1990). the selection of higher GA3 yield strain I. In: National Agro-Biotech Item (Bio-08-02-02).
2. **Yan Fanggui, He Zengguo and Li Jilun.** (1994). The status quo of GA4+7 fermentation. *Microbiology*, vol. 21, No. 3.
3. **Yan Fanggui, He Zengguo and Li Jilun.** (1995). The study on GA4+7 fermentation. *Acta Mycologia*, Vol. 14, No. 3.
4. **He Zengguo** (1997) Improving apple quality by spray of GA4+7. In: The guidebook of Applicable Techniques for Little to Medium Cities, pp. 63-65. Chinese Scientific Press, Beijing 1997.
5. **Li Shao-Hua, He Zengguo, Liu Guojie et al** (1997). The effect of "Fruit-Zheng-Xing-Su" on the development of three kinds of apples. *ShanXi Guoshu*, Vol 67, and (1): 7-9.
6. **Li Shao-Hua, He Zengguo, Liu Guojie** (1997). Study on the improvement of apples fruit indexes by spraying "Fruit-Zheng-Xing-Su" in the full bloom phase. *Zhongguo Guo Shu*, Vol. 66, and (3): 1-5.
7. **He, Z., D. Kisla, L. Zhang, C. Yuan, K. B. Green-Church, and A. E. Yousef.** 2007. Isolation and identification of a *Paenibacillus polymyxa* strain that co-produces a novel lantibiotic and polymyxin. Appl. Environ. Microbiol.73:168-178.
8. **Zengguo He, Chunhua Yand, Liwen Zhang and Ahmed Yousef.** 2007. Paenibacillin: a novel lantibiotic with N-terminal acetylation. (Submitted to *Journal of Biological Chemistry*).

SCIENTIFIC CONFERENCE PRESENTATION

1. Zengguo He and Mayra de la Torre (1999). Effects of media composition on submerged conidiation of Trichoderma harzianum IMI 206040. Accepted as SIMPOSIO of IV Congreso Latinoamericano de Biotecnologia y Bioingenieria, 1999 Mexico.
2. Zengguo He, and Mayra de la Torre (1999). Microcycle conidiation of *Paecilomyces fumosoroseus* Pfrd induced by temperature shift in submerged culture. Abstract for IV Congreso Latinoamericano de Biotecnologia y Bioingenieria, 1999 Mexico, P30.
3. Zengguo He, Lihua Zhang, Fengxia Qi and Page W Caufield (2002) Enhanced production of mutacin I and III from *Streptococcus mutans*. The 42nd Interscience Conference on Antimicrobial Agents and Chemotherapy (ICAAC) in San Diego, California, USA.
4. He, Z., D. Kisla and A. E. Yousef. 2005. Novel Antimicrobial Agent of a Food Isolate *Paenibacillus* sp. The105th ASM General Meeting, June 8, 2005, Atlanta, GA. Abstract: O-075.
5. He, Z., L. Zhang, C. Yuan, D. Kisla, and A. E. Yousef . 2006. Identification of a new lantibiotic DF1-peptide from the fermentate of *Paenibacillus polymyxa* DF1. The 46th Interscience Conference on Antimicrobial Agents and Chemotherapy (ICAAC), September 30, San Francisco, CA. Abstract: F1-1875.

EDUCATION

- Ph.D. (1996) Microbiology
- China Agricultural University, College of Biological Science, Beijing, China.

- M.S. (1991) Microbiology
- China Agricultural University (formerly Beijing Agricultural University), College of Biological Sciences, Beijing, China.

- B.S. (1986) Microbiology
- Beijing Agricultural University College of Biological Sciences, Beijing, China

PROFESSIONAL SOCIETIES/MEMBERSHIPS

- American Society for Microbiology
- Mexican Society for Biotechnology
- Chinese Society for Microbiology
- Chinese society for Biotechnology